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Diffuse Senile Plaques Occur Commonly in the Cerebellum in Alzheimer's Disease

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Diffuse senile plaques are characterized by the presence of β protein (βP), also called A4 protein, in a dispersed form and the apparent lack of associated dystrophic neurites or reactive glial cells. They are the most common type of senile plaque found in the cerebral cortex in Alzheimer's disease (AD), Down's syndrome (DS), and normal aging. Here is reported the frequent presence of diffuse senile plaques in the molecular layer of cerebellar cortex in AD. Typical neuritic plaques were never detected in this location, making the cerebellar molecular cortex a useful site for the study of diffuse plaques because diffuse plaques in the cerebral cortex are intermingled with neuritic plaques. Diffuse cerebellar plaques were detected by modified Bielschowsky silver stain in 47 of 100 cases of clinically and pathologically diagnosed AD and in none of 40 aged demented and nondemented controls. They were immunolabeled by antibodies to purified AD meningeal or cortical βP, and to a synthetic βP but not by two antibodies to the carboxyl- and amino-termini of the β protein precursor (βPP), which label a subgroup of cerebral cortical plaques. This latter result suggests that the βP deposited in the cerebellar molecular layer may be derived from a form of the βPP from which the carboxyl and amino terminal regions of the precursor have already been cleaved. Diffuse cerebellar plaques were not recognized by antibodies to neurofilaments, tau, and PHF, all of which detect dystrophic neurites in cerebral cortical neuritic plaques. Also, no association of reactive astrocytes or microglial cells with diffuse cerebellar plaques was observed. Thus, diffuse cerebellar plaques represent multifocal deposits of noncompacted βP that cause little or no morphologic reaction in their microenvironment. (Am J Pathol 1989, 135:309–319)

The use of the modified Bielschowsky silver stain in the study of Alzheimer's disease (AD) and Down's syndrome (DS) demonstrated the frequent presence of silver-positive plaque-like lesions that have no compacted amyloid cores, obvious dystrophic neurites, or reactive glial cells. They are thus distinguished from both "primitive" (neuritic) and "classical" (amyloid core-containing) senile plaques, and are not detected with either Bodian silver or Congo red stains. These lesions have been called "diffuse plaques" by Yamaguchi and coworkers,[1] although others have used the term "very primitive plaque,"[2] "pre-amyloid" deposit[3] or "amorphous non-congophilic plaques"[29] to describe similar if not identical lesions. Whether these plaque types evolve into other types is currently uncertain.

As revealed by the modified Bielschowsky stain, diffuse plaques are by far the most prevalent type of plaque found in AD, DS, and aged normal brains. There is thus some irony in using the older term classical plaque to describe neuritic plaques with prominent compacted amyloid cores, given that such plaques typically are a minority of the total.[1,4]

Diffuse plaques are found in the cerebral cortex, where they are interspersed among other plaque types, and also in subcortical locations such as the caudate, putamen, claustrum, hypothalamus, thalamus, basal forebrain, and brainstem tegmentum. The modified Bielschowsky silver method stains diffuse plaques as focal, granular and/or faintly fibrillar, amorphous lesions ranging in diameter from less than 10 μm to more than 200 μm. They may occur in clusters and seem to cause little alteration in the adjacent neuropil. Nerve fibers traversing these plaques appear to be neither displaced nor distorted, and it is common to find apparently normal neuronal cell bodies and sometimes tangle-bearing neurons within them. As with

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the plaques having Congo red-positive amyloid deposits, diffuse plaques may occur in a perivascular location.

Several investigators[1,3,5–10] have reported that senile plaques showing a range of morphologies in the cerebrum are labeled by antibodies to synthetic and purified β protein (βP). The βP immunostaining in diffuse plaques[1,3,5] is dispersed and not compacted into a core, as it is in classical plaques. It is not yet known if the βP immunoreactivity in diffuse plaques reflects the presence of dispersed amyloid filaments or of βP-immunoreactive material that is not filamentous.

We report here on the presence, distribution, and immunoreactivity of cerebellar diffuse plaques in a large series of AD patients. The cerebellar molecular cortex provides a useful site to study diffuse plaques because neuritic plaques were not detected in this location. In the cerebral cortex, by contrast, diffuse plaques with immunostaining characteristics indistinguishable from those in the cerebellum are intermingled among neuritic plaques.[1,29] This latter observation raises the possibility that diffuse plaques may evolve into neuritic forms. If so, the apparent absence of neuritic plaques in cerebellar molecular cortex suggests that the evolution of βP deposits in the human brain depends on local environmental influences that differ among brain regions.

Materials and Methods

Patient Groups

AD

One hundred brains were received consecutively over an 18-month period from June 1986 to December 1987 from multiple sources as part of an ongoing research program in which one brain half was frozen for biochemical studies and the other half fixed in formalin.[11] There were 46 men and 54 women having an average age of 76 (range 54 to 97). The clinical diagnoses of AD was made by community and academic physicians. No history of cerebellar symptoms or signs was reported for any of the AD patients. Donation of brain tissue was initiated by the family in almost all instances.

Aged Demented Non-AD

The 20 brains in this category were received over a 2-year period from June 1986 to June 1988 as part of the same research program described above. All patients were demented and were clinically diagnosed as AD, but at autopsy none fulfilled histologic criteria for that diagnosis. There were 16 men and four women, with an average age of 75 (range 62 to 85). Neuropathologic diagnoses were as follows: Parkinson's disease, ten; Pick's disease, three; Lobar atrophy without Pick bodies, five,[12] vascular dementia, one; and limbic encephalitis, 1.

Aged Nondemented

Twenty brains from aged nondemented patients without any history of dementia or other neurologic illness were obtained over a 2-year period from June 1986 to June 1988 from the autopsy service of the Brigham & Women's Hospital. There were seven men and 13 women, with an average age of 76 (range 64 to 87). None fulfilled pathologic criteria for AD.

Tissue Processing

Routine Formalin-Fixed Paraffin-Embedded Tissue

Six-μm sections were cut from a cerebellar slice 1 cm parasagittal to the vermis. Medulla, pons, midbrain, basal forebrain, hippocampus, basal ganglia, thalamus, and neocortex from frontal, temporal, parietal, and occipital lobes were also examined in all 100 AD and 40 control cases. Hematoxylin-eosin (H&E) and modified Bielschowsky silver stains were done on every section. Cerebral cortical and cerebellar sections were also stained with Congo red and examined with a Zeiss polarizing microscope. Selected cases were also studied using Thioflavin S.

Frozen Tissue

Cerebellar cortexes from selected cases (two AD cases with numerous diffuse cerebellar plaques on Bielschowsky stain and four AD cases without diffuse cerebellar plaques) was snap-frozen unfixed for immunocytochemical studies. Sections measuring 10 μm were cut on a cryostat, and immunostained after 10-minute acetone fixation.

Briefly Formalin-Fixed Paraffin-Embedded Tissue

Cerebellar cortexes from six other cases (three AD cases with cerebellar plaques on Bielschowsky stain, two AD cases without cerebellar plaques, and one case of Parkinson's disease without cerebellar plaques) were fixed for one hour in 10% neutral-buffered formalin, and kept in Tris saline at 4 C until paraffin embedding.[13]

Diagnostic Criteria

Histologic criteria for the diagnosis of AD were those outlined by the National Institutes of Health/American Association of Retired Persons (NIH/AARP) Research Workshop on the Diagnosis of Alzheimer's Disease.[14] All cases fulfilling histologic criteria for AD, regardless of the patient's age, were labeled as AD; there was no separate category for senile dementia of the Alzheimer type. Our histologic criteria for Parkinson's disease and for vascular dementia were those previously described.[11]

Antibodies And Techniques Used for Immunostaining

Routine formalin-fixed paraffin-embedded, briefly formalin-fixed paraffin-embedded, and acetone-fixed frozen brain sections were immunostained according to standard techniques.[15] Briefly formalin-fixed paraffin-embedded sections proved to be optimal for experiments in which all antibodies were employed. Tris saline buffer [0.05 M Tris, pH 7.5, 150 mM NaCl] was used. Endogenous peroxidase activity was blocked by incubating the sections with 0.3% hydrogen peroxide in methanol for 30 minutes. Primary antibody was applied overnight at 4 C. The avidin:biotinylated horseradish peroxidase complex system (Vectastain ABC, Vector Laboratories, Burlingame, CA) was used to label bound primary antibody and diaminobenzidine to visualize bound-reaction product. Identically prepared cerebral cortical sections from the same cases were immunostained at the same time as the cerebellar sections to provide positive controls for all antibodies and for the lectin RCA-1. Tissue sections in which primary antibody or lectin was omitted were uniformly negative. Adjacent sections (6 μm in thickness) were used to compare results using the various antibodies. Alternating sections were immunostained with βP-reactive and non-βP antibodies. Results were regarded as meaningful when the non-βP antibody (tau, PHF, neurofilament, or GFAP antibodies) or lectin (biotinylated RCA-1) failed to show abnormal focal immunolabeling in the area corresponding to focal βP-positive deposits in the adjacent sections on both sides.

The following antibodies, which were used for immunostaining were, unless otherwise noted, raised and previously characterized by us.

βP-Reactive Antibodies

Antibody FI (1:300) is a polyclonal antibody to HPLC-purified AD meningovascular amyloid protein[9]; antibody A (1:300) is a polyclonal antibody to HPLC-purified AD cerebral cortical amyloid protein[16]; and antibody $\beta_{1\text{-}28}$ (1:200) is a polyclonal antibody to the 28 residue synthetic βP.[6] Antibodies F1, A, and $\beta_{1\text{-}28}$ all recognize synthetic $\beta_{1\text{-}28}$P as well as 4-7 kDa protein bands on Western blots of preparations of HPLC-purified amyloid protein from AD meninges[9] and cortex.[16]

βPP-Reactive Antibodies

Antibody anti-C_1[27] (1:175) was raised to a synthetic peptide of the 20 C-terminal amino acids of the βPP (residues 676 to 695 of βPP_{695}). Anti-$\beta PP_{45\text{-}62}$ (1:175), a gift of S. Younkin,[28] was raised to a synthetic peptide of βPP residues 45-62 close to the amino terminus.

Tau/PHF-Reactive Antibodies

Antibody P (1:250) is a polyclonal antibody to SDS-isolated paired helical filaments of AD neurofibrillary tangles[17]; antibody DJ (1:500) is a polyclonal antibody recognizing tau and MAP-2 proteins[18]; and monoclonal antibody 5E2 (tissue culture supernatant used 1:20) is specific for tau protein.[13]

Neurofilament Antibodies

SMI-34 (1:10,000) is a monoclonal neurofilament antibody produced by Sternberger-Meyer Inc (Jarrettsville, MD). The neurofilament monoclonal antibody RT-97[30,31] (1:250) was a gift of B. Anderton.

Other

The biotinylated lectin, Ricinus communis agglutinin I (RCA-I) (Vector Laboratories) was used as a marker for microglial cells.[8] Briefly formalin-fixed tissue sections were incubated with diluted biotinylated lectin (1:250) overnight and developed identically to the antibody-treated sections, except that the biotinylated secondary antibody step was omitted. RCA-1 labeling was completely absorbed by pretreatment of tissue sections with 0.2 M β-D-galactose in Tris saline for 4 hours at room temperature.[8] GFAP (1:1000) antiserum was the gift of Dr. D. Dahl.

Antibody Absorptions

Immunoreactivity of antibody A on AD cerebral and cerebellar tissue sections, and on dot blots (1000 purified AD amyloid cores/dot) was absorbed by preincubation of the diluted antiserum overnight using purified AD amyloid cores[16] (20 μg/λ antibody A). To ensure that this absorption was specific, an aliquot of diluted antiserum was simultaneously incubated with fractions from identically

prepared aged non-AD brain. Immunoreactivity of antibody A incubated with these control fractions was completely preserved. Immunostaining by antibody $\beta_{1\text{-}28}$ was absorbed by preincubation of the diluted antiserum overnight with $\beta_{1\text{-}28}$ synthetic peptide (20 μg/λ antibody L). Immunostaining by unrelated antisera (DJ, GFAP) was not affected by incubation with synthetic $\beta_{1\text{-}28}$, fractions of purified AD amyloid cores, or identically prepared fractions from aged control brains.

Electron Microscopy (EM)

The following formalin-fixed cerebellar tissue from autopsy brains was examined by EM. 1) An AD case (aged 75) showing large numbers of diffuse cerebellar plaques at the light microscopic level (LM) (six grids); 2) an aged matched nondemented control subject showing no cerebellar diffuse plaques by LM (nine grids); and 3) a nondemented young control (aged 40) with no detected cerebellar plaques at the LM level (six grids). Areas to be studied were cut from the formalin-fixed cerebellar slice directly opposing that which had been embedded in paraffin and examined with the Bielschowsky silver stain. Regions were selected from the AD case that corresponded to those showing the highest density (average of 16 diffuse plaques/mm^2 microscopic field) of molecular layer plaques in the Bielschowsky-stained section; in the same section, there was less than 1 Purkinje layer amyloid core/mm^2 microscopic field.

Results

Cerebellar Plaques

Two types of plaque-like βP-immunoreactive deposits were seen in AD cerebellar cortexes. 1) Diffuse plaques in the molecular layer and 2) compacted amyloid cores in the Purkinje and granular cell layers.

Diffuse plaques in the molecular layer of cerebellar cortex were commonly seen in AD but were not detected in aged controls. Small-to-moderate numbers of diffuse plaques were detected by modified Bielschowsky silver stain in the molecular layer of the cerebellar cortex in 47 of 100 cases of clinically and pathologically diagnosed AD, but in none of the 40 aged demented and nondemented controls.

As visualized by the modified Bielschowsky silver stain, diffuse cerebellar plaques ranged in size from smaller than 10 μm to elongated lesions extending the width of the molecular layer. They were often found in clusters, separated by stretches of uninvolved cortex, and a variable proportion were clearly perivascular in location. No dystrophic neurites were seen associated with them, and normal appearing nuclei were found within them. Subpial silver positive deposits of a similar histologic character were also seen.

As with diffuse plaques in the cerebrum, those in the cerebellum were not visualized by H&E or Congo red stains. Using thioflavin S, they were not seen in routinely fixed specimens, but were detected as diffusely stained amorphous lesions without associated dystrophic neurites in tissue sections that had been only briefly formalin fixed (see Materials and Methods).

When present, diffuse cerebellar plaques were detected in far smaller numbers than diffuse plaques in the cerebrum of the same cases. They differed from diffuse cerebral plaques in the same brain in that, in certain planes of section, they exhibited a prominent directional orientation that often appeared to be aligned with, or sometimes orthogonal to, the parallel fibers of the molecular layer. This alignment of the silver-stained material also affected the overall shape of the lesions, sometimes resulting in a streaked rather than spherical configuration. When cut in cross section or obliquely, they appeared granular.

Diffuse cerebellar plaques in AD were immunolabeled by βP-reactive antibodies, but not by two antibodies to C- and N-termini of the βPP. Antibodies A and F1, (raised against HPLC-purified fractions of AD cortical and meningeal βP, respectively) and also antibody $\beta_{1\text{-}28}$ (raised against a synthetic βP) labeled both the diffuse cerebellar plaques and the subpial deposits in frozen sections and briefly formalin-fixed, paraffin-embedded sections of AD cerebellar cortex. Absorption studies using antibodies A and $\beta_{1\text{-}28}$ showed complete abolition of immunolabeling when the antisera were absorbed with their specific antigens. Formic acid pretreatment[19] was not necessary for this labeling and did not produce any definite enhancement of staining in the briefly formalin-fixed sections. The immunostaining patterns correlated closely with the silver-positive lesions, with βP-reactive antibody labeling, or with both as seen in adjacent sections. No diffuse plaques were identified in the non-AD cases by antibodies A, F1, and $\beta_{1\text{-}28}$.

No cerebellar diffuse plaques or subpial deposits were detected using either the carboxyl-terminus antibody anti-CI, or an antibody raised to a synthetic peptide close to the N-terminus, anti-$\beta PP_{45\text{-}62}$. Control sections of amygdala from the same cases showed labeling of a subgroup of plaques by each of these antibodies.

Diffuse cerebellar plaques were not found to be associated with reactive neurites or glial cells. Reactive neurites were never demonstrated in the vicinity of βP-immunolabeled diffuse plaques, when adjacent sections (sepa-

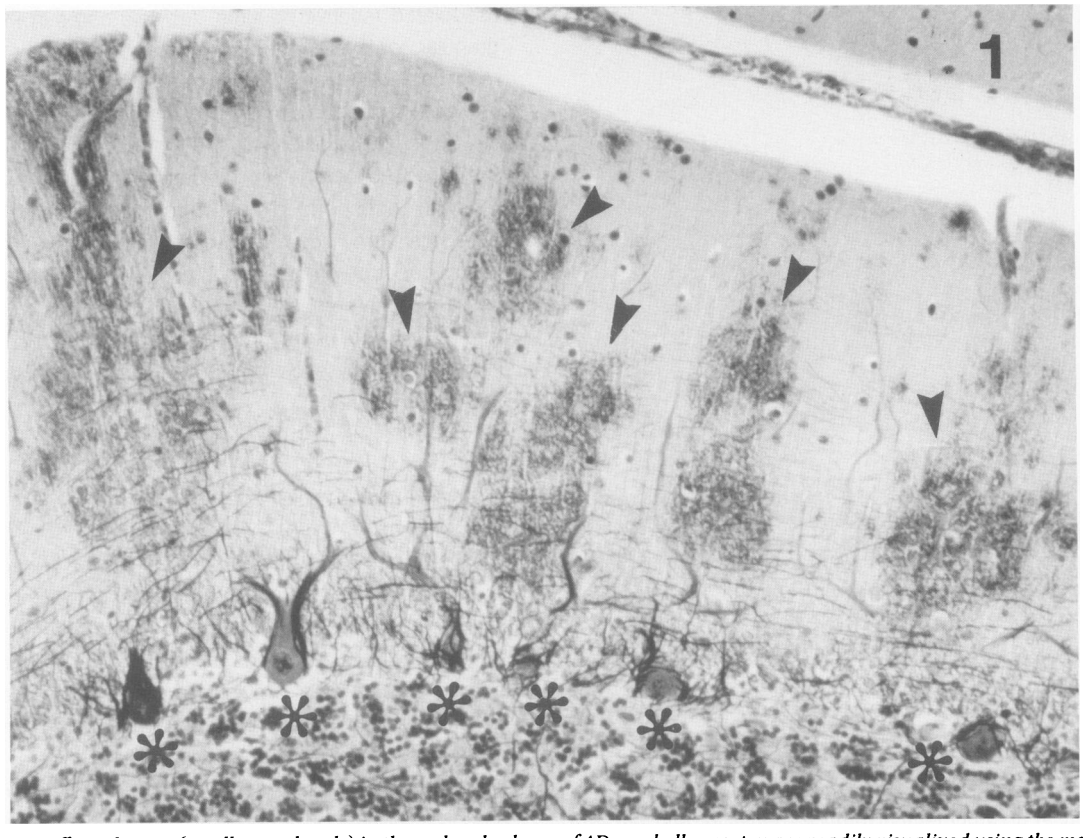

Figure 1. *Diffuse plaques (small arrowheads) in the molecular layer of AD cerebellar cortex are readily visualized using the modified Bielschowsky stain. Purkinje cells (*) show no apparent morphologic changes in the region of diffuse plaques. Cerebellar diffuse plaques were not detected using Congo red (×200).*

rated by 6 μm) were examined using antibodies to neurofilaments, tau or PHF. The two neurofilament antibodies, RT-97[30,31] and SMI-34, labeled basket cell axons investing Purkinje cell bodies as well as parallel fibers in adjacent molecular cortex of tissue from AD and controls. The tau monoclonal 5E2 and PHF antibody P showed no immunoreactivity on cerebellar sections; neither of these antibodies labels tau protein in its normal distribution in the tissue preparations examined.[13,18] Tau/MAP polyclonal antibody DJ labeled large Purkinje cell dendrites in AD cases and controls. Concurrently immunostained sections from AD cerebral cortex demonstrated plaque neurite and neurofibrillary tangle labeling by all of the neurofilament, tau, and PHF antibodies.

Neither reactive astrocytes nor microglial cells showed an association with diffuse cerebellar plaques. The GFAP antibody demonstrated abundant fibers in the molecular cortex of AD cases and controls that were largely perpendicular to the Purkinje layer, as well as reactive astrocytes that were most obvious in white matter. The molecular layer GFAP-positive fibers showed no apparent alteration in the area of βP-labeled diffuse plaques as seen on adjacent sections, some of which were as large as 200 μm in greatest diameter. Senile plaques with reactive astrocytes were readily detected using this antibody on concurrently immunostained sections of AD cerebral cortex. The lectin RCA-1, in cerebellar tissue from AD and control, labeled small cells with delicate branching processes morphologically consistent with microglial cells; blood vessels were also labeled. No apparent association of microglial cells with diffuse cerebellar plaques could be detected. Numerous plaques with surrounding RCA-1-positive microglial cells were seen in sections of AD cerebral cortex that were stained simultaneously.

There was no apparent correlation between the presence or absence of diffuse cerebellar plaques and the degree or distribution of cerebellar amyloid angiopathy. Of the 47 AD patients with diffuse cerebellar plaques, 13 showed no amyloid angiopathy in the same cerebellar section. There were 34 AD cases (six with severe cerebellar amyloid angiopathy) and seven aged controls with cerebellar amyloid angiopathy in which no diffuse plaques were seen.

An EM correlate of diffuse cerebellar plaques was not identified. EM examination of AD cerebellar cortex rich in diffuse plaques failed to demonstrate collections of amy-

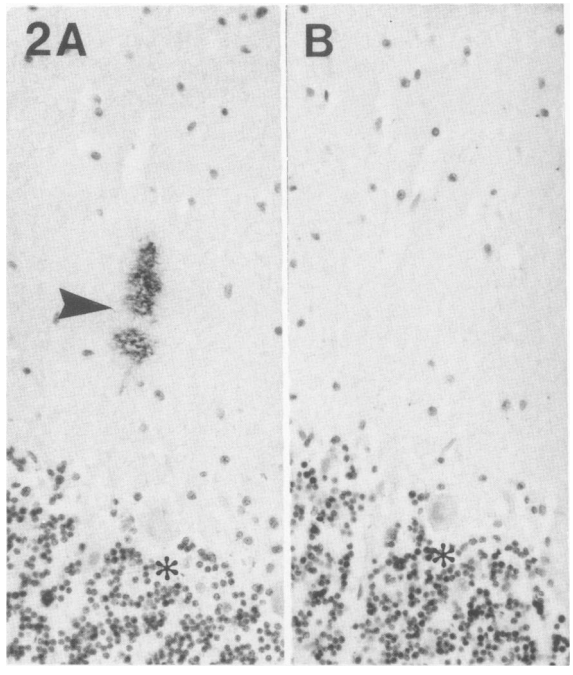

Figure 2. *The immunolabeling of cerebellar molecular layer diffuse plaques was specific and absorbable using βP-reactive antibodies A, F1, and L.* **A**: *Diffuse plaques (large arrowhead) labeled by antibody A preabsorbed with control brain fractions (see Materials and Methods).* **B**: *A corresponding area of an adjacent section showing complete absorption of immunostaining by preabsorption of antibody A with AD amyloid core fractions. *, Purkinje cell (×333).*

loid fibrils that could correlate with the Bielschowsky-stained and βP-immunoreactive lesions observed at the light microscopic level. Examination did reveal occasional electron dense bundles of tightly adherent 10- to 13-nm unbranched fibrils, which were difficult to resolve. These were seen in the molecular layer of cerebellar cortex, running orthogonal to, or sometimes in the same orientation as, the parallel fibers. Some of these fibrils were unequivocally within cell processes. However, similar bundles of fibrils were seen to a lesser degree in aged normal controls lacking diffuse cerebellar plaques in sections examined by LM.

Compacted amyloid cores were present in the Purkinje and granular cell layers of AD cerebellar cortex, and were immunolabeled by βP-reactive antibodies. Scattered isolated amyloid cores were seen in the Purkinje layer and that portion of the granular cell layer closest to the Purkinje layer in AD cases using Congo red, Thioflavin S, and Bielschowsky silver stains.

These dense deposits in Purkinje and granular cell layers were specifically labeled by antibodies A, F1, and $\beta_{1\text{-}28}$ but not by the C- and N-terminal βPP antibodies anti-CI and anti-$\beta PP_{45\text{-}62}$, respectively. They ranged in size from 10 to 50 μm. Adjacent sections examined with neurofilament-, tau-, or PHF-reactive antibodies did not show associated reactive neurites. Amyloid cores in Purkinje, granular cell layers, or both were not detected by the βP-reactive antibodies in non-AD cases.

EM examination demonstrated a dense collection of 6 to 10 nm amyloid fibrils in the Purkinje cell layer of the examined AD cerebellum. This amyloid deposit was believed to correspond to the Congo red-, Thioflavin S-, and βP-positive amyloid cores seen by LM in the same case, and as reported by others.[21,32] Amyloid deposits in blood vessels of the cerebellar cortex and meninges were also seen. They were ultrastructurally similar to amyloid deposits in arteries, arterioles, and capillaries in the cerebral hemispheres.

Cerebellar Vascular Amyloid

Cerebellar amyloid angiopathy, to at least a minimal degree, was seen in 65 of 100 AD cases. In the cerebrum, at least rare vessels with amyloid angiopathy were detected in all 100 AD cases using Congo red stain. The degree of cerebellar amyloid angiopathy reflected but never exceeded the extent of amyloid angiopathy in maximally involved areas of the cerebral hemisphere. When present, it was usually seen in the arteries of the subarachnoid space, but intraparenchymal pericapillary amyloid was also seen in some of the more severely affected cases. Amyloidotic vessels were also detected in the cerebellum in six of 20 aged demented non-AD cases (ten of these 20 cases had at least slight amyloid angiopathy in the cerebrum). Amyloid angiopathy in the cerebellum was present in only one of 20 aged nondemented controls; 11 of these 20 cases had at least trace amounts of amyloid angiopathy in the cerebrum.

The βP-reactive Antibodies A, F1, and $\beta_{1\text{-}28}$ labeled cerebellar and cerebral vascular amyloid in AD, aged demented non-AD, and aged normal subjects. There was excellent correspondence with adjacent Congo red-stained sections. Pericapillary amyloid deposits were not associated with any neuritic response in the neuropil of the molecular layer in so far as could be detected by Bielschowsky silver stain or immunostaining of adjacent sections using neurofilament-, tau-, or PHF-reactive antibodies.

The carboxyl- and amino-terminal βPP antibodies, anti-C_1[27] and anti-$\beta PP_{45\text{-}62}$[28] did not label vascular amyloid under the immunostaining conditions employed.

Discussion

Cerebellar plaques of the diffuse type were present only in AD cases in our series, and in none of 40 aged controls.

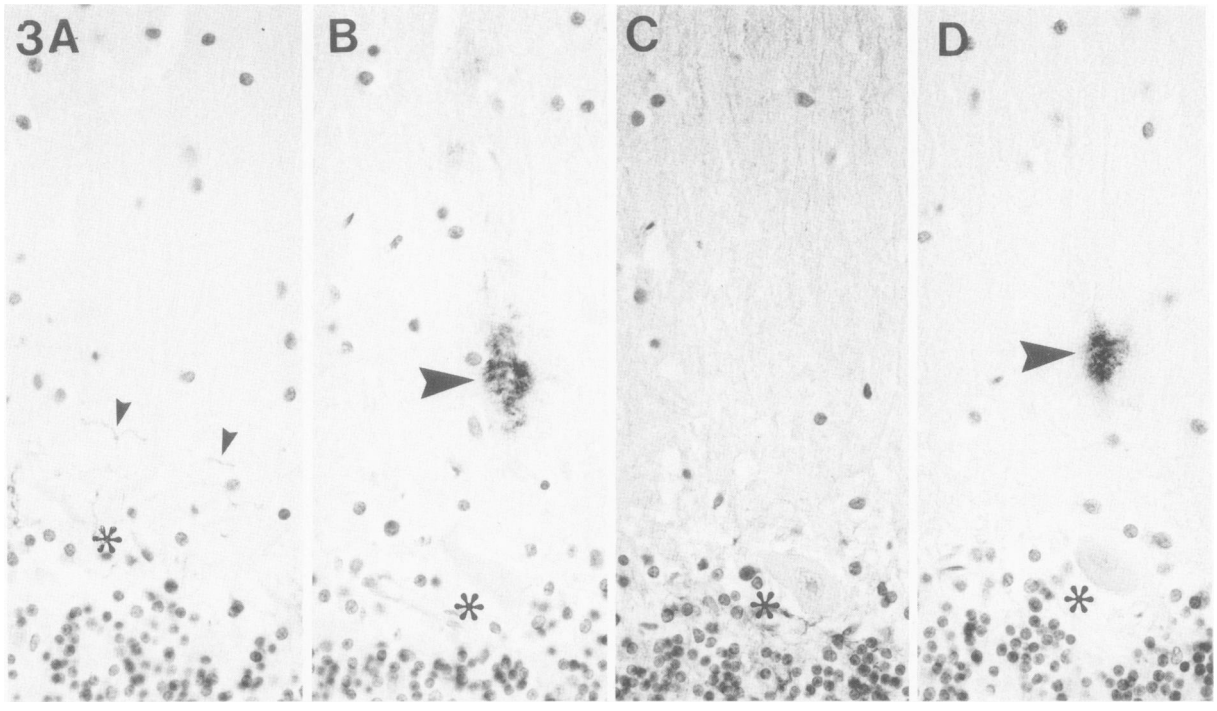

Figure 3. *Neither neurofilament-positive dystrophic neurites nor reactive glial cells or fibers were associated with AD cerebellar diffuse plaques.* **A** *through* **D** *show corresponding regions of four adjacent AD cerebellar sections.* **A:** *Neurofilament monoclonal antibody RT-97 immunolabeled basket cell axons (small arrowheads) in the vicinity of Purkinje cells (*) in AD as well as controls, but showed no focal abnormal alteration in the area corresponding to a diffuse plaque (large arrowhead) shown in the adjacent section* **B:** *which was immunolabeled by Antibody A.* **C:** *GFAP antibody labeled abundant fine processes, predominantly aligned perpendicularly to the Purkinje cell layer in the molecular layer of AD and control cerebellar sections. No focal alteration in the GFAP pattern was detected in the area of the diffuse plaque immunolabeled by antibody A in the preceding adjacent section* **(B)** *and antibody F1 in the next adjacent section* **(D)** *(large arrowhead). *, Purkinje cell (only the dendrites are seen in A) (×400).*

They were observed in low-to-moderate numbers in the molecular cortex of 47 of 100 cases of clinically and pathologically diagnosed AD. Yamaguchi and coworkers[20] found them in four of six AD brains. We conclude that diffuse cerebellar plaques occur only in brains with sufficient numbers of cerebral plaques to fulfill histologic criteria for the diagnosis of AD.

The cerebellar molecular layer lesions observed in cases of AD are definable as diffuse plaques because they appear both to lack dystrophic neurites and to have βP immunoreactivity that is dispersed rather than compacted into a core. Their location and failure to stain with Congo red distinguish them from the compacted amyloid cores seen in Purkinje and granular cell layers of cerebellum. Typical neuritic plaques were never demonstrated in the cerebellar molecular cortex in our study. This location thus is useful for the study of diffuse plaques and contrasts with the cerebral cortex in which diffuse and neuritic plaques are intermingled. A panel of antibodies to neurofilaments, tau, and PHF, which immunolabel cerebral plaque neurites, failed to identify neurites in association with the diffuse plaques of the cerebellar molecular cortex. Likewise, no focal astrocytic or microglial response could be demonstrated using a GFAP antibody and the lectin, RCA-1, respectively.

The diffuse cerebellar plaques were immunolabeled by antibodies to synthetic βP as well as βP purified from AD brain, without the need for formic acid pretreatment.[19,20] They were not recognized by two antibodies to the carboxyl- and amino-termini of βPP, which immunostain a subgroup of cerebral cortical senile plaques. The presence of carboxyl- and amino-terminal βPP epitopes in some cerebral plaques suggests that proteolytic processing of the full length βPP molecule could occur in a highly localized fashion at the site of plaque formation for these plaques.[27,28] An alternative explanation, however, could be that carboxyl- and amino-terminal epitopes of βPP may appear at the site of plaque formation, possibly in neurites,[29] as a secondary phenomenon. The complete lack of immunostaining of cerebellar diffuse plaques by these βPP amino- and carboxyl-terminus antibodies raises the possibility that the βPP deposited in cerebellar molecular layer plaques may arrive with its amino and carboxyl termini already cleaved. Similar mechanisms could apply to the deposition of βP in cerebral vessels; we found no immunocytochemical evidence to indicate that

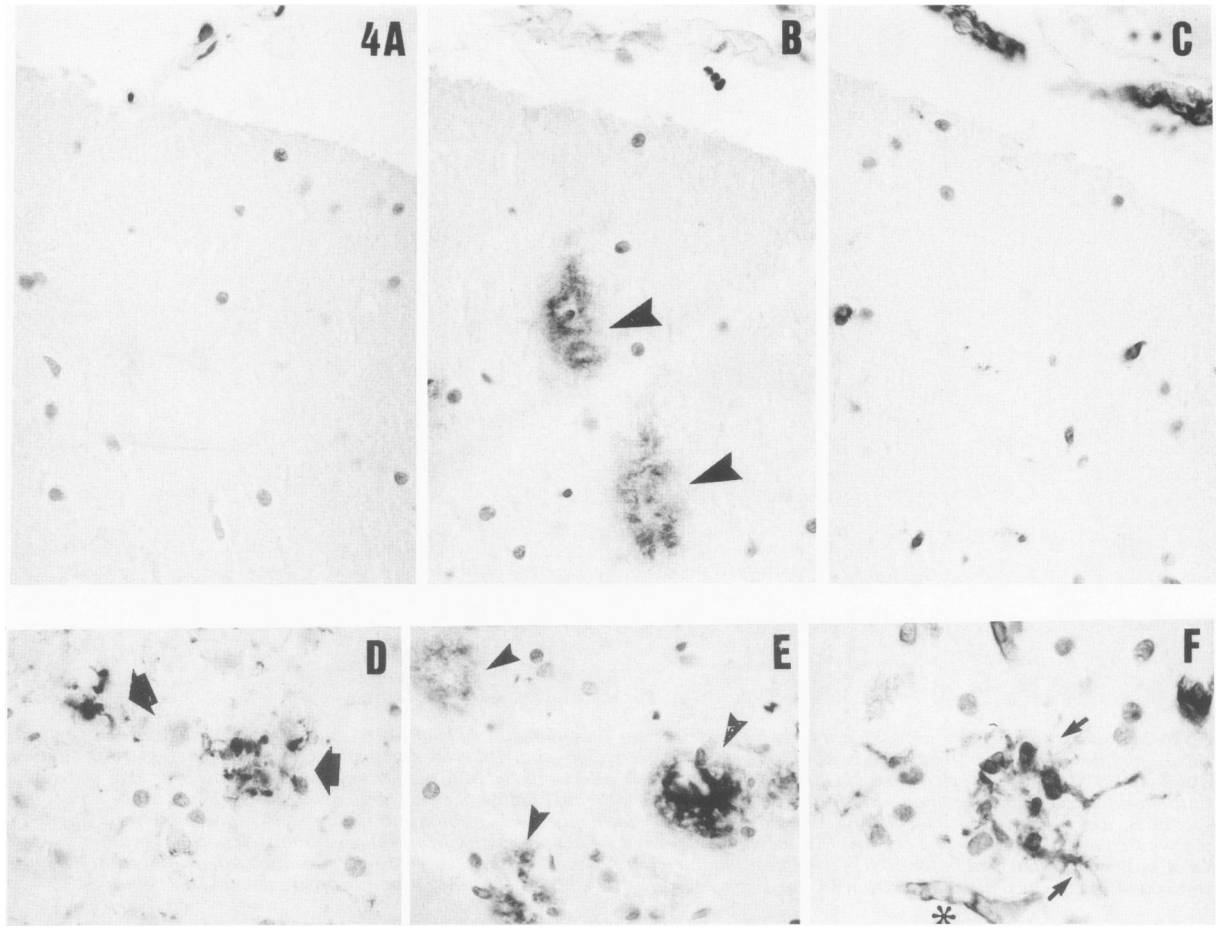

Figure 4. *The tau monoclonal antibody, 5E2, and the lectin, RCA-1, did not demonstrated dystrophic neurites or focal clusters of microglial cells, respectively, in the vicinity of cerebellar diffuse plaques, although each labeled plaques in cerebral sections from the same AD brain immunostained at the same time.* **A** *through* **C** *show corresponding areas of adjacent AD cerebellar cortical sections.* **D** *through* **F** *show different regions of AD amygdala from the same brain immunostained simultaneously.* **A:** *Under the immunostaining conditions employed, 5E2 labels dystrophic neurites and neurofibrillary tangles, but not tau in its normal axonal distribution. No immunostaining is present in the area of cerebellar molecular layer cortex corresponding to two diffuse plaques labeled by antibody A in the adjacent section* **(B)**. *In the amygdala* **(D)**, *5E2 recognizes dystrophic plaque neurites (large arrows).* **B:** *Antibody A labels diffuse plaques in the cerebellum (large arrowheads), and in the amygdala* **(E)** *plaques (small arrowheads) of various morphologies.* **C** *and* **F:** *The lectin, RCA-1, labels small cells with fine processes, consistent with microglial cells as well as blood vessels in sections from AD and controls.* **C:** *No focal clustering of microglial cells is apparent in the region of the diffuse plaques immunolabeled in the adjacent section* **(B)** *whereas a plaque in amygdala* **(F)** *contains several microglial cells (small arrows). A small blood vessel (*) nearby is also labeled (A through E, ×400; F, ×450).*

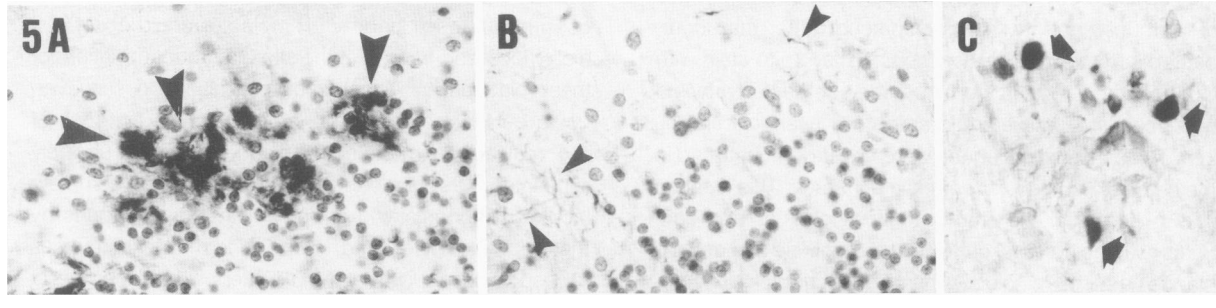

Figure 5. *Amyloid cores were detected in the Purkinje cell layer in AD cases using Congo red stain, Thioflavin S, and βP immunostain. No dystrophic neurites were identified in the region of these lesions.* **A:** *Dense deposits (large arrowheads) immunostained by antibody A in the Purkinje cell layer of an AD cerebellum.* **B:** *The corresponding area of the adjacent section immunostained with the neurofilament monoclonal antibody RT-97 shows labeling of basket cell axons (small arrowheads) but no abnormal reaction in the region of the amyloid deposit seen in the adjacent section (A). RT-97 labels dystrophic neurites (arrows) in a plaque in the amygdala from the same brain processed simultaneously* **(C)** *(A and B, ×400; C, ×900).*

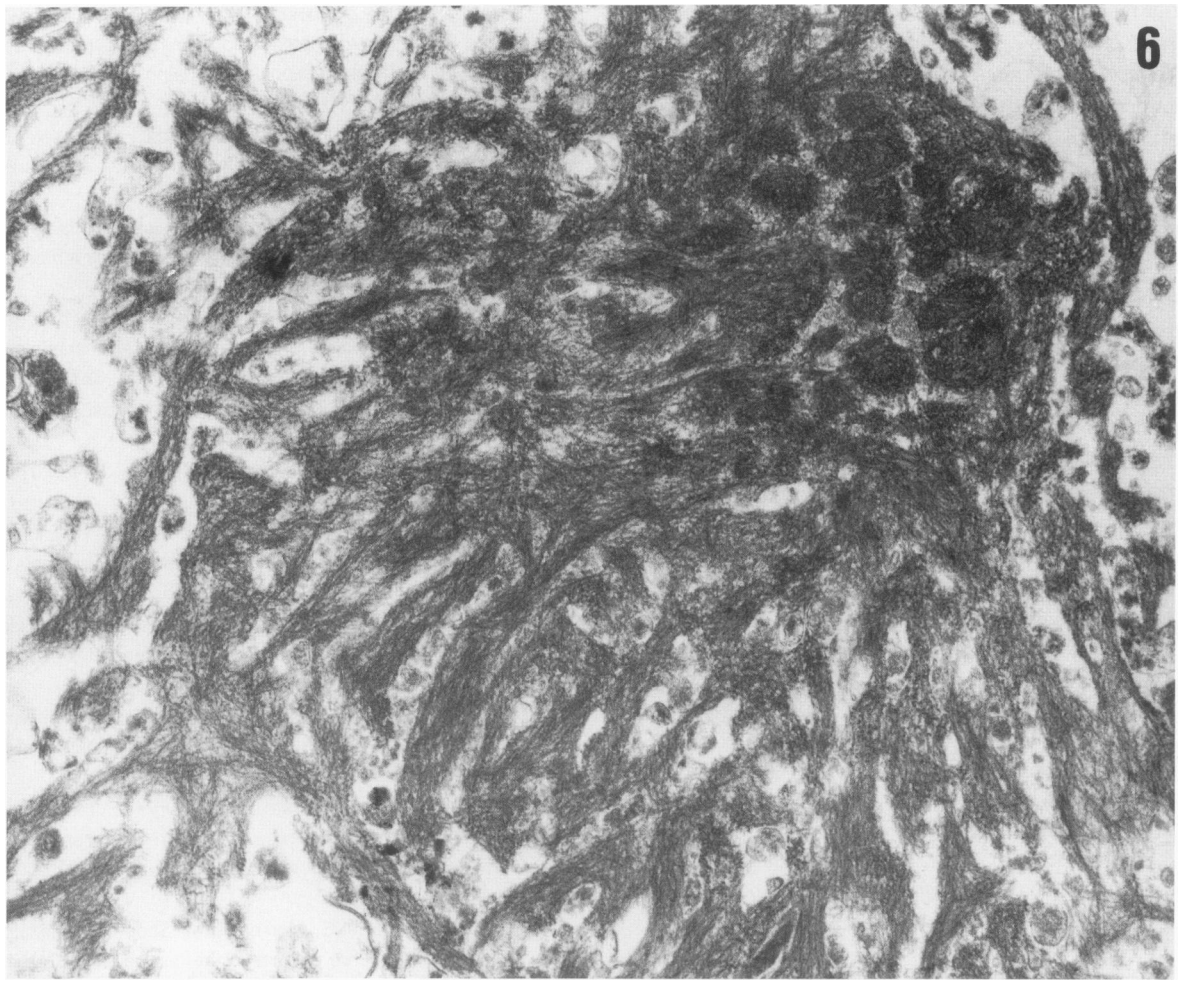

Figure 6. *Dense collections of amyloid fibrils were seen in the Purkinje cell layer of an AD cerebellum examined by electron microscopy, most likely corresponding to the amyloid cores seen in this layer by light microscopy (eg, Figure 5) (×25,000).*

vascular amyloid contains epitopes for carboxyl- or amino-terminal regions of βPP. Further studies employing a range of antibodies to regions throughout the βPP may help clarify these issues.

It is still unknown whether diffuse plaques in the cerebrum, cerebellum, or both contain βP in the form of amyloid fibrils, or as nonfibrillar deposits. The diffuse, noncellular pattern of βP immunolabeling suggests that the protein is located extracellularly. However, ultrastructurally we have so far been unable to identify amyloid fibrils in regions of AD cerebellar molecular cortex known to contain large numbers of diffuse plaques, despite that tissue preservation was adequate to identify one of the less numerous amyloid cores in the Purkinje cell layer of the same cerebellum. Further electron microscopic studies are needed to resolve this issue.

Determination of the ultrastructural correlate of cerebral and cerebellar diffuse plaques may also shed light on the issue of whether diffuse plaques are in fact a very early form of senile plaque. If so, it is interesting that, in the cerebellar molecular layer, further progression to neuritic forms rarely, if ever, occurs. An alternative view could be that diffuse plaques are a separate plaque type, which does not evolve further, in either the cerebral or cerebellar cortex. This view would suggest that there are distinct mechanisms involved in the deposition of βP in diffuse and neuritic plaques.

The source of the βP that is deposited in cerebellar plaques and blood vessels is unknown. We did not detect morphologic changes in any of the cells or processes in areas of molecular cortex containing abundant diffuse plaques. Messenger RNA for the βP precursor is known to be present in Purkinje cells[23] but has not so far been reported in other cerebellar cells. In contrast to the neurons in the basal forebrain[24] and locus ceruleus,[25] there is as yet no evidence of increased βP precursor mRNA levels in Purkinje neurons in AD compared with control brains. The βP-reactive antibodies used in this study do

not recognize the βP precursor in normal neurons; Purkinje cells, as well as other neurons, were unlabeled. Thus, we were unable to detect, either by morphologic or immunocytochemical observations, cerebellar cells that showed evidence of being responsible for producing the βP in diffuse cerebellar plaques. Alternatively, the perivascular location of some diffuse cerebellar plaques raises the possibility of a non-CNS (blood) origin for the βP, and the frequent observation of subpial βP-immunoreactive deposits in both cerebral and cerebellar cortex suggests that βP, its precursor, or both could enter brain tissue from the cerebrospinal fluid. In this latter regard, Palmert et al[2,26] have recently detected near full-length forms of the βP precursor lacking the carboxyl-terminus in human cerebrospinal fluid. However, the origin of the βP and the mechanisms by which it is selectively deposited in neural tissue remain to be elucidated.

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Since submission of this manuscript, Suenaga and coworkers (J Neuropathol Exp Neurol 1989, 48:336) have reported positive immunolabeling of cerebellar diffuse plaques by antibodies to ubiquitin, but not tau or neurofilament antibodies. We have confirmed these findings in our own laboratory. The ultrastructural localization of the ubiquitin reaction product has not yet been determined and remains an important question in the interpretation of this observation.